A versatile software–programmable microfluidic platform for automated biology. Electronic Supplementary Information

Luis M. Fidalgo and Sebastian J. Maerkl*

Received Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
First published on the web Xth XXXXXXXXXX 200X
DOI: 10.1039/b000000x

1 Methods

1.1 Fabrication protocol

Microfluidic devices were fabricated using multilayer soft-lithography[1]. Photolithography masks were designed using Clewin (WieWeb) and written directly on AZ precoated chromium substrates using a Heidelberg DWL200 laser writer. Molds were fabricated on 4" silicon wafers using a Karl Suss MA6 mask aligner. Two photoresists were used to generate either square (SU8) or round (AZ) channel profiles. Table S1 summarizes the materials and thicknesses used for the structures comprised in the three layers of the device.

Layer	Structure	Material	Thicknes (μm)
1	Routing	SU8	40
2	Valves	SU8	30
	Vias	SU8	35
	Control	AZ	14
3	Flow	AZ	10
	Multiplexer	SU8	20

Table S1: Materials and thicknesses used for each structure found in the three layers of the PMD

Devices were molded in poly(dimethyl siloxane) (PDMS) (RTV 615, General Electric). The fabrication protocol was adapted from that previously described by Hansen *et al*[2]. Molds were treated with Chlorotrimethylsilane (TMCS, Sigma) to aid release of the PDMS replicas. Layer 1 was cast to an approximate thickness of 3 cm, degassed and baked for 75 min at 80 °C. Layer 2 was spin coated at 1600 rpm and left at room temperature for 30 min before baking for 30 min at 80 °C. The wait time at room temperature before baking helped ensuring the planarity of the *via*–containing PDMS film. Layer 1 was aligned to layer 2 and both were baked together for 30 min at 80 °C. Layer 3 was spin coated at 2500 rpm and baked for 30 min at 80 °C. After punching access holes (21G diameter), bonded layers 1 and 2 were aligned to layer 3 and baked together for 75 min at 80 °C. Finally, after another punching step, finished devices were cleaned and bonded to a glass slide using oxygen plasma (Femto type, Diener).

1.2 Device architecture

In order to individually route each core valve while maintaining channel and valve dimensions, we placed the routing channels and the valves on two separate layers interconnected through microfluidic *vias*[3]. This allowed maintaining a valve area of 100x100 μm^2 that ensured operation at moderate pressures ($\sim$25 psi) with short actuation times ($\sim$100 ms). Figure S1 shows cross–section schematics of the device in the core and multiplexer regions with details of the routing channels, *vias*, valves and flow and multiplexer channels included in the three layers. Core valves push down on flow channels for closing whereas multiplexer valves push up on control channels.

We observed that when closing core valves fluid was occasionally displaced between nodes in an uncontrolled manner. Fluid displacement is inherent to this type of valve, but in a closed network design such as ours it can induced cross–talk between nodes. To prevent this flow, and the possible contamination it could induce, we used fluidic capacitors to accommodate the fluid displaced when closing core valves. The capacitors consisted of square spaces vertically above each node into which the PDMS membrane could deflect, avoiding flow between nodes. Figure S2 contains a schematic of a node and the corresponding capacitor when the adjacent core valves are either open or closed. It also shows micrographs of the same time–point within a patterning routine in two different devices, with and without fluidic capacitors.

1.3 Device operation

Control and input fluids were injected in the device through metal pins (0.30 mm ID, 0.65 mm OD) connected to segments of tygon tubing (0.020" ID, 0.060" OD) that were pressurized using distribution manifolds connected to house compressed air. Microfluidic valves were actuated by computer controlled solenoid manifolds (Pneumadyne Inc.) that were driven using custom software written in Visual Basic. The principle

Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2011

for device operation consisted in addressing valves using the multiplexer before determining their state using the core input valve. Closing the multiplexer before successively addressing multiple valves allowed storing the state of each valve (Fig. 2 a–e). The software used to operate the devices allowed input at multiple levels of complexity, ranging from determining the state of single valves to patterning node contents using a single instruction. More advanced software routines were developed for each application, namely: active mixing, surface immunoassays and cell culture.

1.4 Image acquisition

Device images and videos were obtained using a color CCD camera (AVT Pike F-145C) connected to an inverted microscope (Nikon Eclipse Ti–S). Scanning fluorescence micrographs of antibody patterns were obtained using a custom–modified microarray scanner (Array Worx). Cell culture time–lapse images were acquired using an Andor Ixon+DU888 EMCCD camera attached to an automated inverted microscope (Nikon Eclipse Ti–E). Illumination light was provided by a LED lamp (CoolLED) set to 100% power at 535 nm. Filters used corresponded to Texas red dye (Ex 560 nm / Em 645 nm). Images were taken at 60x magnification (40x air objective combined with the built–in 1.5x magnification) with 1 s exposure. They were analyzed using Image J to provide mean intensity values that were averaged for each pair of duplicate experiments (Fig. 4f). Final fluorescence images were acquired using 20x magnification to allow visualization of chamber pairs (Fig. 4f inset).

2 Results

2.1 Software–reconfigurable channel implementation

Fluid delivery into the core network is based on the implementation of channels defined by opening a sequence of valves connecting the network entrance and exit. Video S1 shows a real–time sequence of this process. The software input for channel implementation requires the user to select the nodes to be included in the channel. A simple algorithm connects the network entrance with each selected node and the channel exit by opening the valves placed between them.

In this example each valve opening, accounting addressing and opening times, requires 100 ms. The time required to replace the fluid in the channel depends on the channel geometry and the pressure in the flow channel. In this case the exchange takes place in 6 s and consumes approximately 100 nl of solution. The pressures in the flow, control and multiplexer channels were 8, 30 and 55 psi respectively.

2.2 Automated patterning and display

The program for patterning node contents in the network is based on the channel implementation concept described above. It creates rectangular channels to iteratively replace the contents of each node with either a red dye or water until the displayed pattern matches the input given by the user. This input is a sequence of letters to be displayed, which are built pixel by pixel and then stored for a given time before constructing the next pattern. Video S2 shows a sequence of this process recorded at 5 frames per second and played back at 30 frames per second, therefore accelerated 6 times. The pressures in the flow, control and multiplexer channels were 5, 25 and 55 psi respectively. Writing each pixel required a total of 7.5 seconds, divided into creating the required channel (2.5 s) and replacing the contents of the node (5 s).

2.3 Active mixing and formulation

Implementing ring channels while using core valves to induce peristaltic flow provides a simple method for active mixing within the network. Ring mixers of multiple geometries can be created at any network location by means of software. Video S3 shows an image sequence where multiple ring mixers are implemented sequentially, using them to actively mix a colored dye ($Fe(SCN)_3$). The sequence was recorded at 5 frames per second and is played back at 30 frames per second, therefore accelerated 6 times. Initially, a ring spanning 12 nodes is implemented and homogenized before three rings involving 4 nodes each are created at different locations. In a separate experiment, we used ring mixers to create an array of dye concentrations by loading an increasing number of nodes with dye in 3 separate 4–node rings and mixing them in parallel (video S4, real time).

2.4 Cell trapping and culture

Cell culture experiments were performed using wild type yeast (BY4741, MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) and a mutant expressing m–cherry under the control of the PHO5 promoter (BY4741; trp1Δ::P_{PHO5} – mCherry – Kan). We received these strains from Arun S. Rajkumar from the LBNC, EPFL. Liquid cultures were prepared by inoculating samples of 3 ml of Synthetically Defined medium (Sigma) that were incubated overnight and diluted 1:2 in fresh medium 4 h before their use. Standard culture medium contained 10 mM inorganic phosphate, whereas induction medium contained no added inorganic phosphate. Culture was conducted at 30 °C.

In order to trap cells at network nodes, firstly a channel path traversing the node to be loaded was implemented (Fig. 4a, Fig. S5a). Then a valve contiguous to the node was closed and manually slowly re–opened by lowering the pressure on the control input. After re–establishing the flow through the node,

This journal is © The Royal Society of Chemistry [year]

Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2011

the pressure was slowly increased, partially closing the valve and effectively creating a sieve that allowed medium to flow but trapped yeast cells at the selected node (Fig. 4b, Video S5, real time). The final seeding density could be adjusted by choosing the cell density of the injected culture, the speed of the flow and the seeding time. In our experiments, flowing the suspension at 5 psi for a few seconds seeded approximately 30–40 cells per node.

The protocol used to culture both strains under different conditions needed to ensure correct compartmentalization of the cells in their respective nodes while maintaining stable media compositions over extended periods. To enable compartmentalized perfusion we used nodes contiguous to those containing cells as perfusion chambers. The basic culture concept consisted of alternatively replenishing the medium in the perfusion chambers while closing the culture chambers and allowing diffusion between culture and perfusion chambers while preventing cross–talk with adjacent cultures. Since there were two separate conditions on the device, these alternating steps were performed simultaneously: while one section was replenished the other was perfused and vice versa (Fig, 4c, Fig. S5b-c). The period of the culture cycle was one minute, which ensured efficient replenishment as well as sufficient diffusion of nutrients between the perfusion and culture chambers. Cells were cultured for 9 h before induction started (Fig. 4d-e, Video S6). Induction was quantified using fluorescence imaging during a 15 hour period (Fig. 4f). During this total culture time of 24 hours the device showed no signs of wear or deterioration.

References

1 M. A. Unger, H.-P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**, 113–116.

2 C. L. Hansen, M. O. A. Sommer and S. R. Quake, *Proc Natl Acad Sci U S A*, 2004, **101**, 14431–14436.

3 E. Kartalov, C. Walker, C. Taylor, W. Anderson and A. Scherer, *Proc Natl Acad Sci U S A*, 2006, **103**, 12280–12284.

Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2011

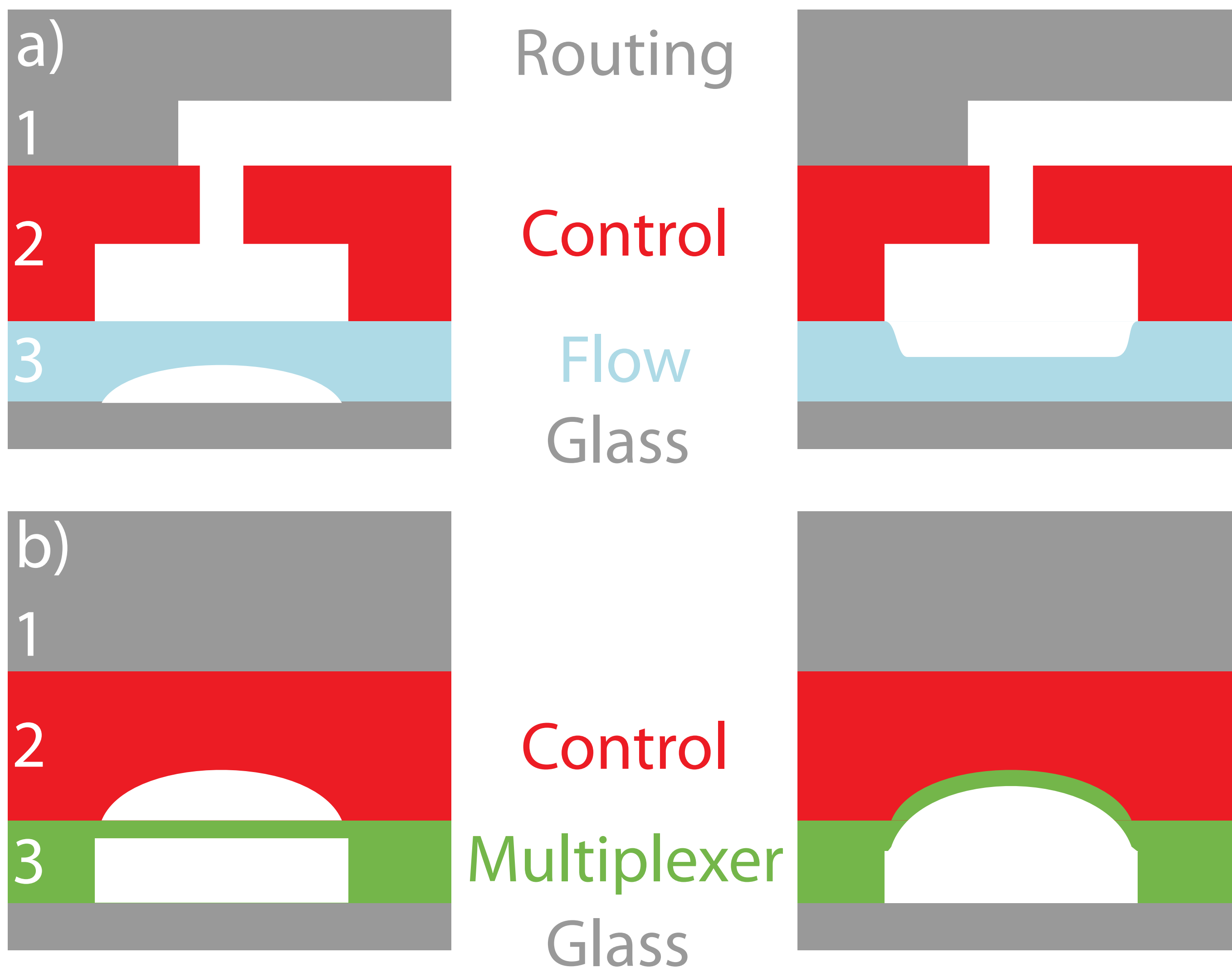

Figure S1: Cross–section schematics of the three layers of the device in the core **(a)** and multiplexer **(b)** regions. In the core region, routing channels are aligned with *vias* connected to PDMS valves that close down on flow channels. In the multiplexer region the control channels that feed onto the routing channels can be closed by pressurizing the multiplexer below.

This journal is © The Royal Society of Chemistry [year]

Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2011

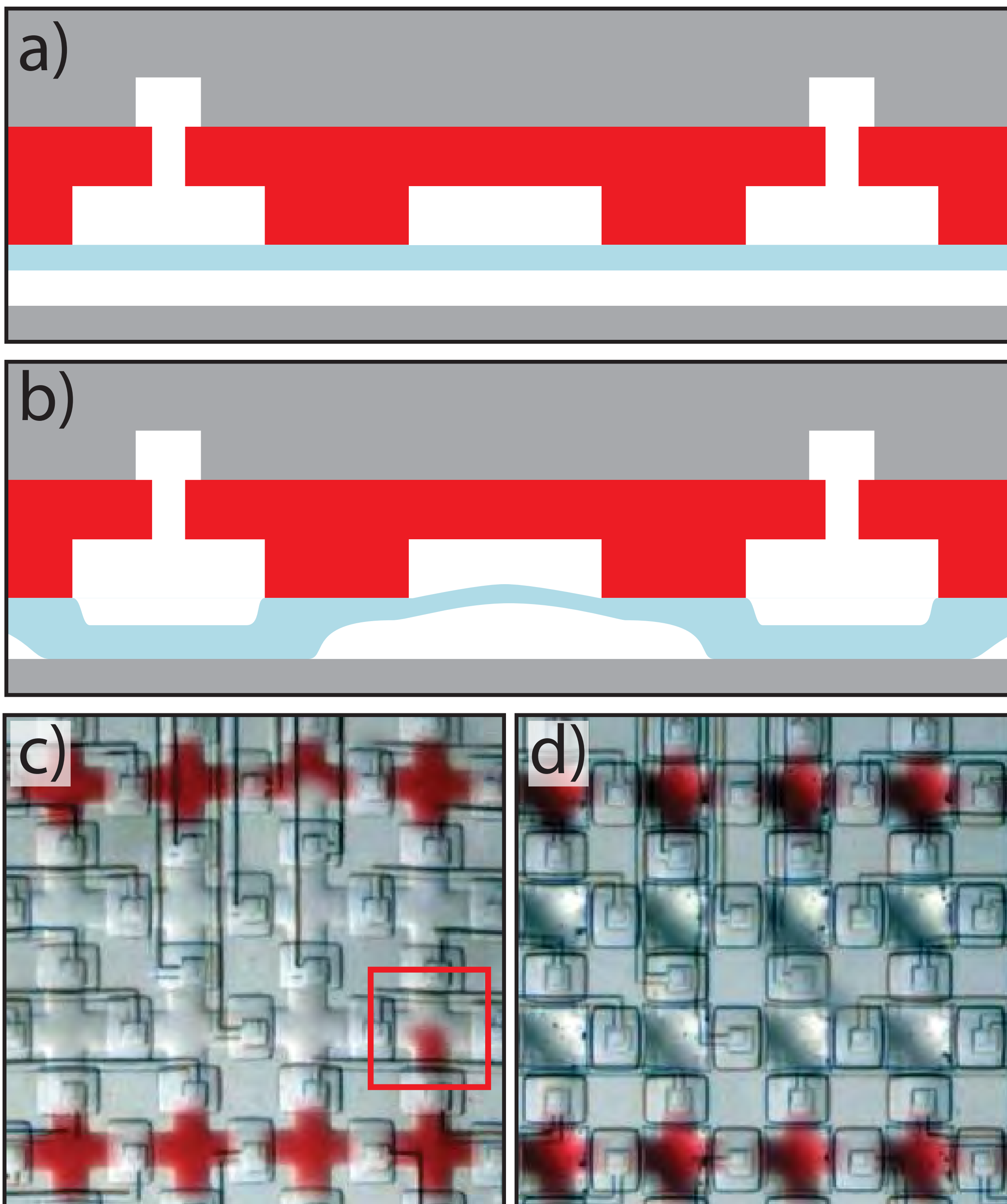

Figure S2: **(a)** Cross–section schematic of the device showing the fluidic capacitor above a network node and two adjacent valves. **(b)** When the valves are pressurized the membrane above the node can deflect upwards preventing unintentional flow between nodes. **(c)** Micrograph of a PMD without fluidic capacitors in the process of creating a dye pattern. Closing the core valves after dye loading causes unintentional flow between two nodes. **(d)** This flow does not appear during the creation of the same pattern in a device featuring a capacitor at each node.

Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2011

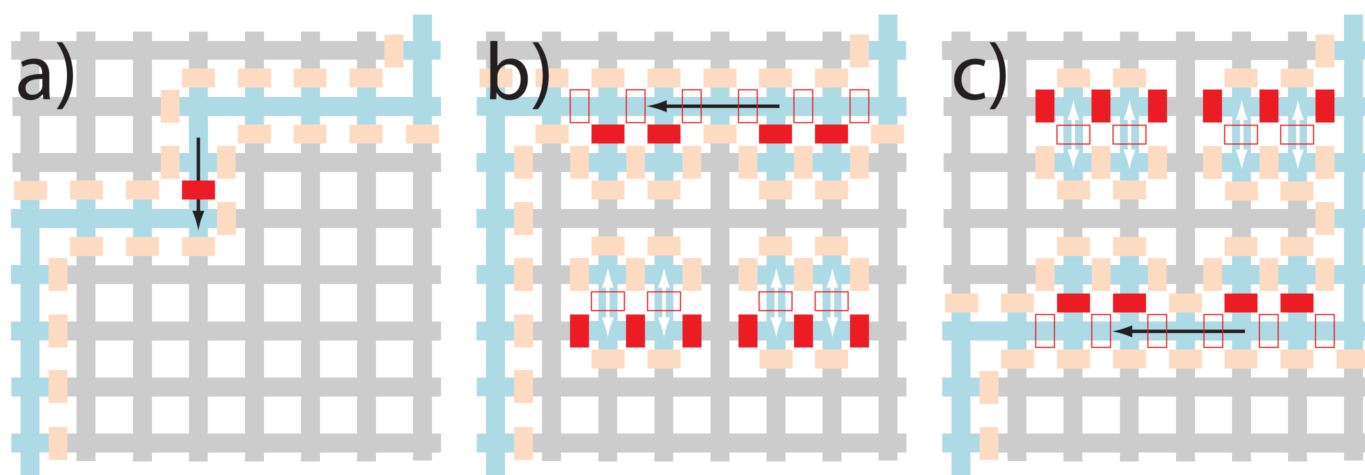

Figure S3: Schematics of the channels implemented on the PMD for cell trapping and culture. The flow channels and active chambers are shown in blue whereas the active valves are shown in red; closed valves are solid, open valves are outlined. Valves surrounding the active channels and chambers are shown in pink, the rest of the network is shown in grey. **(a)** Cell trapping is achieved by flowing a cell suspension through a partially closed valve adjacent to the node to be loaded. **(b, c)** Cell culture is divided in two alternating steps: medium replenishment and perfusion. Medium is replenished by flowing media through perfusion chambers while separating them from culture chambers (top row in **(b)**, bottom row in **(c)**). Once the perfusion chambers are filled with fresh media, culture and perfusion chambers are communicated so nutrients can diffuse between them (bottom row in **(b)**, top row in **(c)**). In order to maintain compartmentalization, culture and perfusion chambers are separated laterally while diffusion occurs.

This journal is © The Royal Society of Chemistry [year]

Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2011